Amendments to the Specification:

Please delete the paragraph on page 6, lines 12-21 and replace it with the following paragraph:

Figure 5 shows inhibition of the M1 protein-induced HBP release by fibrinogen derived peptides and antibodies to CD18. *Panel A*: Human plasma was incubated with peptides Gly-Pro-Arg-Pro **(SEQ ID NO: 2)**, Gly-His-Arg-Pro **(SEQ ID NO: 3)** (100 μg/ml), or buffer alone for 15 min at 37°C. Clotting was initiated by the addition of thrombin and the clotting time was determined. *Panel B*: M1 protein was added to whole human blood (1 μg/ml) followed by the addition of different amounts of Gly-Pro-Arg-Pro **(SEQ ID NO: 2)**, Gly-His-Arg-Pro **(SEQ ID NO: 3)**, antibody mAB IB4 to CD18, or antibody AS88 (directed against human H-kininogen). After 30 min of incubation at 37°C, cells were centrifuged and the amount of HBP in the supernatants was determined. Data are expressed as percent of HBP release induced by M1 protein alone, and the bars represent means ± SD of 3 experiments, each done in duplicate.

Please delete the paragraph on page 12, lines 18-29 and replace it with the following paragraph:

The second component comprises isolated fibrinogen or a functional variant thereof. Fibrinogen is a soluble plasma protein which is converted to insoluble fibrin in the blood by the action of the enzyme thrombin. This contributes to the formation of a blood clot. Fibrinogen is composed of six peptide chains. These are arranged in two identical subunits, each composed of an Aα, a B∃ and a γ chain, joined by disulphide bonds. Streptococcal M protein binds to fibrinogen (Kantor, 1965, J. Exp. Med., 121, 849-859) with high affinity (Åkesson et al., 1994, Biochem. J., 300, 877-886; Berge et al., 1997, J. Biol. Chem., 272, 20774-20781). Fibrinogen also binds to PMNs via $\exists_2$ integrins (Altieri, 1999, Thromb. Haemost., 82, 781-786). The binding site for the $\exists_2$ integrin Mac1 has been mapped to the N-terminal region of the A∀ chain of fibrinogen. In addition, the unique sequence KQAGDV **(SEQ ID NO: 11)**, which is found at the C-terminal end of the (chain, is essential for integrin binding.

Please delete the paragraph on page 24, line 28 to page 25, line 21 and replace it with the following paragraph:

Reagents. Neutrophil Isolation Medium (NIM) was purchased from Cardinal Associates Inc. (Santa Fe, NM). RPMI 1640 medium with Glutamax I (trade mark), Minimum Essential Medium (MEM) with Earle's salts and L-glutamine, fetal bovine serum, and penicillin (5000 units/ml) / streptomycin (5000 µg/ml) solution were purchased from Life Technologies (Täby, Sweden). Ionomycin and formyl-methionyl-leucyl-phenylalanine (fMLP) were obtained from Calbiochem (La Jolla, CA). The acetoxymethyl ester of N,N'-(1,2-ethanediylbis(oxy-2,1-phenylene))bis(N-(carboxymethyl)) (BAPTA), and ProLong® Antifade Kit were from Molecular Probes (Eugene, OR). 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) was from Merck (Whitehouse Station, NJ). Streptococcal cysteine proteinase (SpeB) zymogen was purified from the medium of AP1 bacteria by ammonium sulfate precipitation (80 % w/v) followed by fractionation on S-Sepharose (Berge et al., 1997, J. Biol. Chem., 272, 20774-20781). Recombinant M1 protein, fragments A-S and S-C3, and protein H were obtained by expression in E. coli and purified as described earlier (Åkesson et al., 1994, Biochem. J., 300, 877-886; Berge et al., 1997, J. Biol. Chem., 272, 20774-20781). Recombinant human HBP was produced using the baculovirus expression system in Sf9 insect cells (Invitrogen Corp., Carlsbad, California) and was purified as described (Laemmli, 1970, Nature, 227, 680-685). Lipoteichoic acid (LTA), hyaluronic acid (HA), and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MO). Mouse mAB 2F23C3 and rabbit antiserum (409A) to recombinant HBP were prepared and purified as described earlier (Lindmark et al., J. Leukoc. Biol., 66, 634-643) and peroxidase-conjugated goat anti-rabbit IgG was from Bio-Rad Laboratories (Richmond, CA). Peptides H-2935 (Gly-Pro-Arg-Pro **(SEQ ID NO: 2)**) and H-2940 (Gly-His-Arg-Pro **(SEQ ID NO: 3)**) were purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Fluanison/fentanyl and midazolam were from Janssen Pharmaceutica, Beers, Belgium and Hoffman-La Roche, Basel, Switzerland.

Please delete the paragraph on page 28, line 27 to page 29, line 4 and replace it with the following paragraph:

RT-PCR - RT-PCR was conducted with GeneAmp/PerkinElmer RNA PCR kit according to the manufacturer's protocol. Briefly, total RNA (500 ng) in water was heated (65°C, 10 min), chilled on ice, and reverse transcribed (20 min, 42°GG GTT GTT GAG AA 3′ **(SEQ ID NO: 4)** derived from the genomic sequence (NM 001700) of human HBP), 1 U/µl RNase inhibitor, and 2.5 % de-ionized formamide. After denaturation (5 min, 99°C), samples were amplified in PCR buffer (1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1µM primer, 2.5% de-ionized formamide, and 0.05 1 U/µl *Taq* polymerase) for 20-35 cycles with annealing between 50 and 60°C and extension at 72°C, using a PerkinElmer/GeneAmp PCR system 2400. Products were analyzed by agarose gel electrophoresis (1% gels).

Please delete the paragraph on page 29, lines 5-19 and replace it with the following paragraph:

Animals - Adult male mice (approximately 30 g) of the C57BL/6 strain were used. Animals were anaesthetized with equal parts of fluanison/fentanyl (Hypnorm 10, 0.2 mg/ml) and midazolam (Dormicum, 5 mg/ml) diluted 1:1 with sterile water (dose: 0.2 ml / mouse i.m.). The anaesthesia was supplemented with inhalation of 2% isoflurane. All animal experiments were approved by the regional ethical committee. Mice were given an intravenous injection of 100 µl of a solution containing 150 µg/ml M1 protein. Alternatively, 100 µl of a solution containing 150 µg/ml M1 protein and 4 mg/ml Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** or Gly-His-Arg-Pro **(SEQ ID NO: 3)** were intravenously injected. As control vehicle alone was applied via the same route. 30 min after injection, mice were sacrificed and the lungs were removed. Alternatively, 100 µl of a bacteria solution (2 x 10^9 AP1 bacteria/ml in the presence or absence of 400 µg Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** or Gly-His-Arg-Pro **(SEQ ID NO: 3)**) were injected together with 0,9 ml of air into the dorsal region of the mouse. After 30 min, mice were given an intravenous injection of 100 µl of a solution containing PBS or 2 mg/ml Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** or Gly-His-Arg-Pro **(SEQ ID NO: 3)**, respectively. Six hours after infection, mice were sacrificed and the lungs were removed.

Please delete the paragraphs on page 35, line 30 to page 37, line 8 and replace them with the following paragraphs:

Human fibrinogen binds to PMNs via β_2 integrins (Altieri, 1999, Thromb. Haemost., **82**, 781-786) and for CD11c/CD18 the binding site was mapped to the NH_2-terminal region of the Aα chain of fibrinogen. A peptide derived from this region (Gly-Pro-Arg-Pro; **SEQ ID NO: 2**)), has been shown to block adherence of TNF-stimulated PMNs to fibrinogen-coated surfaces, while other peptides from the same region, including Gly-His-Arg-Pro **(SEQ ID NO: 3)**, had no effect (Loike et al., 1991, Proc. Natl. Acad. Sci. USA, **88**, 1044-1048). Furthermore, it was demonstrated that antibodies against β_2 integrins inhibit the binding of fibrinogen to activated PMNs, and among these antibodies a monoclonal antibody (IB4) directed against the common β-chain of integrins, was the most potent (Loike et al.,1991, Proc. Natl. Acad. Sci. USA, **88**, 1044-1048). Platelet-induced activation of PMNs was also found to be dependent on the interaction between CD11c/CD18 and the Aα chain of platelet-expressed fibrinogen (Ruf and Patscheke, 1995, Br. J. Haematol., **90**, 791-796). As shown for the binding of fibrinogen to PMNs, platelet-induced activation was also inhibited by the Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** peptide and by antibodies to CD11c, whereas the Gly-His-Arg-Pro **(SEQ ID NO: 3)** peptide had no effect. These reports indicate that the binding of PMNs to immobilized fibrinogen (for instance on coverslips or platelets) involves the β_2 integrins leading to an activation of PMNs. Interestingly, Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** not only inhibits the binding of fibrinogen to β_2 integrins, but it also prevents clot formation (Laudano and Doolittle, 1980, Biochemistry, **19**, 1013-1019), and Figure 5A shows that Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** completely blocked thrombin-induced coagulation of normal plasma, while Gly-His-Arg-Pro **(SEQ ID NO: 3)** did not influence the clotting time. It should be emphasized that Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** prevents fibrin-fiber formation by binding to the thrombin exposed polymerization sites of the fibrin molecules (Spraggon et al., 1997, Nature, **389**, 455-462). Thus, the effect of Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** on clot-formation is not integrin-dependent. The influence of the two peptides on the interaction between M1 protein and fibrinogen was tested in a competitive ELISA. However, none of the peptides had an effect in these assays (data not shown).

The Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** and Gly-His-Arg-Pro **(SEQ ID NO: 3)** peptides, as well as antibodies to the β_2 integrins (IB4), were also tested for their ability to interfere with the M1 protein-induced secretion of HBP. As shown in Figure 5B, the addition of Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** to human blood blocked the mobilization of HBP by M1 protein in a dose dependent manner, and also antibody IB4 directed against the common β-chain of integrins impaired the release. The control substances, Gly-His-Arg-Pro **(SEQ ID NO: 3)** and an unrelated antibody to H-kininogen, did not influence HBP secretion (Fig. 5B). The effect of Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** on M1 protein-induced PMN aggregation was confirmed by scanning electron microscopy analysis. Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** inhibited the aggregation of purified PMNs in a mixture of plasma and M1 protein. In contrast, Gly-His-Arg-Pro **(SEQ ID NO: 3)** had no effect on the aggregation of PMNs. These results support the notion that M1 protein-fibrinogen complexes activate PMNs through β_2 integrin ligation, which triggers the release of HBP. This mechanism appears to be similar to the previously described antibody-mediated cross-linking of CD11b/CD18 that mimics adhesion-dependent receptor engagement causing a massive release of HBP from PMNs (Gautam et al., 2000, J. Exp. Med., **191**, 1829-1839).

Please delete the paragraphs on page 37, line 11 to page 39, line 18 and replace them with the following paragraphs:

So far, HBP has only been identified in humans and pigs (Flodgaard et al, 1991, Eur J. Biochem, 197, 535-547). Before mouse experiments were performed, we investigated whether an HBP homologue is also present in the mouse. To this end, bone marrow cells from mice were isolated and the existence of a murine HBP homologue was demonstrated by RT-PCR analysis and Western blot analysis. RT-PCR amplification of RNA prepared from bone marrow cells was carried out using a primer set derived from the human HBP sequence. Western blot detection was carried out after electrophoresis of human HPB and murine bone marrow lysate immunostained with antibodies against human HBP. A series of animal experiments was then conducted with anaesthetized mice. Three mice received M1 protein i.v. (15 μg/animal); three were treated with a mixture of M1 protein (15 μg/animal) and peptide Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** (400 μg/animal); three with a mixture of M1

protein (15 μg/animal) and peptide Gly-His-Arg-Pro **(SEQ ID NO: 3)** (400 μg/animal); and three with vehicle alone. Thirty minutes after administration the breathing of mice injected with M1 protein or M1 protein plus peptide Gly-His-Arg-Pro **(SEQ ID NO: 3)** was clearly affected as compared to the other mice. The animals were sacrificed and the lungs were removed, stained with hematoxylin and eosin and subjected to light microscopy or analyzed by scanning electron microscopy. A representative lung sample from a mouse injected with buffer only showed intact lung tissue. Lung sections from mice injected with M1 protein, however, demonstrated severe hemorrhage and tissue destruction. These lesions were almost completely prevented when M1 protein was injected together with Gly-Pro-Arg-Pro **(SEQ ID NO: 2)**, even though the tissue remained slightly swollen which is a sign of an ongoing inflammatory reaction. By contrast, application of Gly-His-Arg-Pro **(SEQ ID NO: 3)** could not prevent the M1 protein induced bleeding and tissue destruction. Protein H was injected as a control and analysis of the lung tissue revealed no hemorrhage and the alveoli appeared less swollen. In order to resolve lung lesions at higher magnification, tissue sections were analyzed by scanning electron microscopy. A lung section from a PBS-treated mouse showed no signs of any pulmonary damage. However, injection of the M1 protein resulted in severe leakage of erythrocytes as seen before, but also in the deposition of proteinous aggregates. The morphology of the aggregates resembled the M1 protein-induced amorphous plasma precipitates seen earlier. The lungs of mice injected with M1 protein and Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** contained no precipitates. However, some alveolar swelling and minor leakage of erythrocytes were observed indicating an inflammatory reaction. In contrast, treatment with Gly-His-Pro-Arg **(SEQ ID NO: 3)** did not influence M1 protein-caused lung damage. The injection of protein H did neither cause serious bleeding nor did the tissue appear to be severely inflamed.

In order to quantify the degree of lung affection six randomly chosen lung tissue section from each of the twelve animals were analyzed by electron microscopy, and the ratio of lung area containing protein aggregates versus total lung area was determined. Less than 10% of the lung tissue of animal injected with buffer alone or with M1 protein plus the Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** peptide contained protein aggregates (3 ± 1% and 6 ± 2%, respectively). In contrast, 90% of the lungs of animals treated with M1 protein or a mixture of M1 protein and the Gly-His-Arg-Pro **(SEQ ID NO: 3)** peptide contained protein aggregates (90 ± 2% in

both cases). These animal experiments suggest that M1 protein-fibrinogen aggregates activate PMNs via the β_2 integrins, resulting in massive vascular leakage and deposition of protein aggregates in the lung tissue. The results also show that this pathophysiological effect can be blocked when fibrinogen-induced crosslinking of β_2 integrins is prevented by the Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** peptide.

Gly-Pro-Arg-Pro prevents vascular leakage and lung damage in mice infected with M1 protein expressing *S. pyogenes* bacteria

In a second series of animal experiments, nine mice were subcutaneously infected with M1 protein expressing *S. pyogenes* bacteria. Three mice in each group were treated with peptides Gly-Pro-Arg-Pro **(SEQ ID NO: 2)** and Gly-His-Arg-Pro **(SEQ ID NO: 3)** as described in Material and Methods, respectively, while three mice received no treatment. As a control, three mice were given a subcutaneous injection of PBS. Six hours after infection, animals were sacrificed, lungs removed and examined by scanning electron microscopy. Analysis of blood samples from the animals revealed no occurrence of streptococci, indicating that bacteria had not started to disseminate from the site of infection. Electron micrographs of representative lung tissue sections from these animals were obtained. Recovered lungs from mice that received buffer instead of bacteria showed no signs of pulmonary damage. However, mice that were infected with streptococci were suffering from severe lung lesions indicated by massive infiltration of erythrocytes and fibrin deposition. When infected animals were treated with Gly-Pro-Arg-Pro **(SEQ ID NO: 2)**, the lungs appeared to be much less affected, whereas treatment with Gly-His-Arg-Pro **(SEQ ID NO: 3)** failed to prevent pulmonary damage. Lungs from mice infected with streptococci were further analyzed by immuno-staining electron microscopy by using antibodies against M1 protein. This showed that the M1 protein was found in the infiltrated precipitates. In contrast, no M1 protein staining was observed when lungs from non-infected animals were examined. Taken together, these results suggest that in an infectious model, shedded M1 protein is found in the circulation prior to dissemination of bacteria forming precipitates that deposits in the lungs of infected animals.